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EXAMINER	
CHOWDHURY, IQBAL HOSSAIN	
ART UNIT	**PAPER NUMBER**
1652	

DATE MAILED: 06/20/2006

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/697,419	PATTERSON ET AL.	
	Examiner	Art Unit	
	Iqbal Chowdhury, Ph.D.	1652	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *06 April 2006*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-33* is/are pending in the application.

4a) Of the above claim(s) *5-6, 15-26 and 29* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-4,7-14,27,28 and 30-33* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date *08/04*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

This application is a non-provisional of provisional application of 60/422,467 of 10/30/2002.

The preliminary amendment filed on 1/31/2005 amending claims 1-3, 5, 9, 12-13, 15, 19-21, and 23 and newly adding claims 27-33 is acknowledged. Claims 1-33 are pending and are present for examination.

Applicant's election without traverse of Group I, claims 1-4 and 7-14, drawn to isolated polynucleotide comprising codon-optimized nucleotide sequence of SEQ ID NO: 1 encoding bacterial LuxA protein, expression cassette, and host cell, and TTA, CTA, TTG and CTT to CTG or CTC as species in the response filed on 4/6/2006 is acknowledged.

Examiner notes that there was an error in the previous office action regarding groupings of the claims 27, 28, 30 and 31-33, which are related to nucleic acid and should be with Group I. Therefore, claims 27, 28 and 30-33 will be examined with the elected claims of Group I.

The requirement is still deemed proper and is therefore made **FINAL**.

Claims 5-6, 15-26, 29 are withdrawn from further consideration pursuant to 37 CFR 1.142(b), as being drawn to a nonelected invention, there being no allowable generic or linking claim.

Claims 1-4, 7-14, 27-28, 30 and 31-33 are at issue and are present for examination.

Applicants traverse the election of species codon substitution on the ground(s) that the search for the species recited in Group I would not be unduly burdensome and further arguing that 37 CFR 1.141 states that "more than one species --- not to exceed a reasonable number

examined provided the application also includes an allowable claim generic to all the claimed species. The examiner has agreed that all the species would be examined together.

Priority

Acknowledgement is made of applicants claim for priority of provisional application 60/422,467 of 10/30/2002.

Claim Objections

Claims 1, 3, 9, 13, 27, 28, 30 and 30 are objected to with recitation "LuxA protein" as abbreviations should not be used without at least once fully setting forth what they are used for. Appropriate correction is required.

Claim 32 is objected to with recitation "IRES" as abbreviations should not be used without at least once fully setting forth what they are used for. Appropriate correction is required.

Claims 1, 2, 7, 8, 9, 10-12, 27, and 30-33 are objected to as encompassing non-elected subject matter. Appropriate correction is required.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1-3, 7-13, 27-28 and 30-33 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter, which was not described in the specification in such a way as to reasonably convey to one skilled

in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Claims 1-3, 7-13, 27-28 and 30-33 are directed to a genus of a DNA molecule comprising a codon-optimized nucleotide sequence encoding at least one component of bacterial luciferase system LuxA protein. Claim 7 recites that the codon-optimized nucleotide sequence comprising a regulatory element and claim 8 recites that the said regulatory element is an enhancer sequence. Claims 9 and 10 recite a mammalian cell comprising said codon-optimized nucleic acid sequence and claim 11 recites that said cell is immobilized on a substrate. Claim 12-13 recite that said mammalian cell comprises codon-optimized nucleic acid sequence wherein the nucleic acid sequence encoding LuxA protein. Claims 27-28 and 30 recite that the codon-optimized nucleotide sequence encoding LuxA protein expresses higher level when expressed in mammalian cell under expression-promoting conditions.

In written description guidelines the written description requirement for a claimed genus may be satisfied through sufficient description of a representative number of species by actual reduction to practice, reduction to drawings, or by disclosure of relevant, identifying characteristics, i.e., structure or other physical and/or chemical properties, by functional characteristics coupled with a known or disclosed correlation between function and structure, or by a combination of such identifying characteristics, sufficient to show the applicant was in possession of the claimed genus. A representative number of species means that the species, which are adequately described are representative of the entire genus. Thus, when there is substantial variation within the genus, one must describe a sufficient variety of species to reflect the variation within the genus. The specification teaches the structure of only single

representative species of such DNAs.

Moreover, the specification fails to describe any other representative species by any additional identifying characteristics or properties other than the functionality of the codon-optimized LuxA polypeptide having luciferase activity. Given this lack of description of representative species encompassed by the genus of DNAs used in the claims, the specification fails to sufficiently describe the claimed invention in such full, clear, concise, and exact terms that a skilled artisan would recognize that applicants were in possession of the claimed invention.

Claims 1-3 and 7-13, 27-28 and 30-33 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a codon-optimized nucleotide sequence of SEQ ID NO: 1 encoding LuxA protein from Photorhabdus luminescens, does not reasonably provide enablement for any codon-optimized nucleotide sequence encoding any LuxA protein from any bacteria. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make the invention commensurate in scope with these claims.

Claims 1, 3 and 27 are so broad as to encompass any codon-optimized nucleotide sequence encoding any LuxA protein from any bacteria. Claim 7 recites that the codon-optimized nucleotide sequence comprising a regulatory element and claim 8 recites that the said regulatory element is an enhancer sequence. Claims 9 and 10 recite a mammalian cell comprising said codon-optimized nucleic acid sequence and claim 11 recites that said cell is immobilized on a substrate. Claim 12-13 recite that said mammalian cell comprises codon-optimized nucleic acid sequence wherein the nucleic acid sequence encoding LuxA protein.

Claims 27-28 and 30 recite that the codon-optimized nucleotide sequence encoding LuxA protein expresses higher level when expressed in mammalian cell under expression-promoting conditions.

The scope of the claims is not commensurate with the enablement provided by the disclosure with regard to the extremely large number of codon-optimized nucleotide sequence encoding any LuxA protein having any codon substitution broadly encompassed by the claims. Since the amino acid sequence of a protein determines its structural and functional properties, predictability of which changes can be tolerated in a protein's amino acid sequence and obtain the desired activity requires a knowledge of and guidance with regard to which amino acids in the protein's sequence, if any, are tolerant of modification and which are conserved (i.e. expectedly intolerant to modification), and detailed knowledge of the ways in which the proteins' structure relates to its function. However, in this case the disclosure is limited to the nucleotide and encoded amino acid sequence of only one codon-optimized nucleotide sequence encoding LuxA protein.

While recombinant and mutagenesis techniques are known, it is not routine in the art to screen for multiple substitutions or multiple modifications, as encompassed by the instant claims, and the positions within a protein's sequence where amino acid modifications can be made with a reasonable expectation of success in obtaining the desired activity/utility are limited in any protein and the result of such modifications is unpredictable. In addition, one skilled in the art would expect any tolerance to modification for a given protein to diminish with each further and additional modification, e.g. multiple point mutations or substitutions.

The specification does not support the broad scope of the claims which encompass any

codon-optimized nucleotide sequence encoding any LuxA protein because the specification does **not** establish: (A) regions of the protein structure which may be modified with higher luciferase activity; (B) the general tolerance of LuxA protein to modification and extent of such tolerance; (C) a rational and predictable scheme for modifying any LuxA residues with an expectation of obtaining the desired biological function; and (D) the specification provides insufficient guidance as to which of the essentially infinite possible choices is likely to be successful.

Thus, applicants have not provided sufficient guidance to enable one of ordinary skill in the art to make and use the claimed invention in a manner reasonably correlated with the scope of the claims broadly including any codon-optimized nucleotide sequence encoding any LuxA protein having any codon substitution to SEQ ID NO: 1. The scope of the claims must bear a reasonable correlation with the scope of enablement (In re Fisher, 166 USPQ 19 24 (CCPA 1970)). Without sufficient guidance, determination of any codon-optimized nucleotide sequence encoding any LuxA protein having the desired biological characteristics is unpredictable and the experimentation left to those skilled in the art is unnecessarily, and improperly, extensive and undue. See In re Wands 858 F.2d 731, 8 USPQ2nd 1400 (Fed. Cir, 1988).

Claims 9, 10 and 12-14 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for isolated host cells transformed with the recited nucleic acids does not reasonably provide enablement for host cells within a multicellular animal which have been transformed with the recited nucleic acids. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make the invention commensurate in scope with these claims.

Claims 9, 10 and 12-14 are so broad as to encompass host cells transformed with specific nucleic acids, including cell in *in vitro* culture as well as cells within any multicellular organism. The scope of the claims is not commensurate with the enablement provided by the disclosure with regard to the extremely large number of host cell broadly encompassed by the claims. While methods for transforming cell *in vitro* are well known in the art, methods for successfully transforming cells within complex multicellular organisms are not routine and are highly unpredictable. Furthermore, methods for producing a successfully transformed cell within one multicellular organism are unlikely to be applicable to transformation of other types of multicellular organisms as multicellular organisms vary widely. However, in this case the disclosure is limited to only host cell in vitro.

Thus, applicants have not provided sufficient guidance to enable one of ordinary skill in the art to make and use the claimed invention in a manner reasonably correlated with the scope of the claims broadly including the use of host cells within a multicellular organism for the production of polypeptide. The scope of the claims must bear a reasonable correlation with the scope of enablement (In re Fisher, 166 USPQ 19 24 (CCPA 1970)). Without sufficient guidance, expression of genes in a particular host cell having the desired biological characteristics is unpredictable and the experimentation left to those skilled in the art is unnecessarily, and improperly, extensive and undue. See In re Wands 858 F.2d 731, 8 USPQ2nd 1400 (Fed. Cir, 1988). It is suggested that applicants limit the claims to "An isolated host cell".

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are

such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

The factual inquiries set forth in *Graham* v. *John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 1-3, 8-13, 27-28 and 30 are rejected under 35 U.S.C. 103(a) as being obvious over Szittner et al. (J Biol Chem. 1990 Sep 25; 265(27): 16581-7, see IDS), Mao et al. (Zhonghua Zhong Liu Za Zhi, 2001 Sep; 23(5): 359-62, article in Chinese) in view of Nawotka et al. (WO 03/016839 A2, publication 2/27/2003) or Zolotukhin et al. (US Patent 5,874,304, publication 2/23/1999). Szittner et al. teach LuxA (luciferase) gene from Xenorhabdus (same as Photorhabdus) luminescens, which is 70% identical to SEQ ID NO: 1 of the instant application, wherein the source of LuxA gene also from Photorhabdus luminescens. Szittner et al. also teach the cloning of Lux genes required for expression of luminescence, complete nucleotide sequences of the LuxA gene coding for the alpha subunit of luciferase. Szittner et al. further teach that the luciferase from X. luminescens have a remarkably high thermal stability being stable at 45 degrees C (t 1/2 greater than 3 h) and suggested that the X. luminescens Lux system might be used for application in coupled luminescent assays and expression of Lux genes in

eukaryotic systems at higher temperatures. Szittner et al. do not teach the use of codon-optimized or codon usage of LuxA gene for maximum expression with stability in eukaryotic or mammalian cells.

Mao et al. teach the expression of fused LuxAB gene of bacterial luciferase as a reporter gene in mammalian liver carcinoma cells. Mao et al. also teach cloning bacterial luciferase LuxA and B gene in the mammalian expression vector pcDNA3, wherein the promoter and enhancer is from cytomegalovirus (CMV) and transfected into BEL7402 cell and determined the luciferase activity with standard assay method. Mao et al. do not teach codon-optimized LuxA gene.

Zolotukhin et al. teach a humanized green fluorescent protein (GFP) genes and method of use. Zolotukhin et al. also teach synthetic and humanized versions of GFP genes adapted for high-level expression in mammalian cells especially those of human origin by using base substitution in codons in order to change the codon usage for efficient expression in mammalian cells. Zolotukhin et al. also teach increase number of CTG or CTC leucine encoding codons, increase number of TTC for phenylalanine encoding codons and increase number of ATC isoleucine encoding codons of GFP amino acid sequence. Zolotukhin et al. also teach cloning the modified gene in expression vector and expressing in mammalian cells at higher efficiency.

Nawotka et al. teach the native and modified form of nucleic acid sequence of luciferase gene from Phrixothrix hirtus. Nawotka et al. also teach that native and modified form of luciferase could be used as reporter molecules in host cells and transgenic animals. Nawotka et al. further teach the codon optimization by utilizing codon usage database for expression in human, mouse, candida, and cryptococcus. Nawotka et al. furthermore teach by stating that it is

preferable to change all leucine codons such as TTA, CTA, TTG and CTT to CTG, as CTG is the most used leucine codon in mammalian cells.

It would have been obvious to one of ordinary skill in the art at the time of the invention was made to combine the teachings of Szittner et al., Mao et al. and Zolotukhin et al. or Nawotka et al. to codon-optimize luciferase gene of Szittner et al. including substituting the leucine codon CTG instead of other leucine codons as disclosed by Zolotukhin and Nawotka et al. in order to optimum expression in mammalian cell and to clone the codon-optimized gene in mammalian expression vector under the regulation of promoter/enhancer as disclosed by Mao et al. to use the codon-optimized Lux system in the development a mammalian bioluminescence bioreporter system to be used in medical research and diagnostics applications.

One of ordinary skill in the art would have been motivated to use codon-optimized LuxA gene for mammalian cells in order to maximum expression in that mammalian cells for the efficient and stable enzyme activity in terms of luminescence to be used in medical research and diagnostics applications.

One of ordinary skill in the art would have a reasonable expectation of success because use of codon-optimized gene for over-expression with higher stability in a mammalian cell is customary and widely used in the art.

Claims 31-32 and 33 are rejected under 35 U.S.C. 103(a) as being unpatentable over Szittner et al. (J Biol Chem. 1990 Sep 25; 265(27): 16581-7, see IDS), Mao et al. (Zhonghua Zhong Liu Za Zhi, 2001 Sep; 23(5): 359-62, article in Chinese) in view of Nawotka et al. (WO 03/016839 A2, publication 2/27/2003) or Zolotukhin et al. (US Patent 5,874,304, publication

2/23/1999) as applied to claims 1-3, 8-13, 27-28 and 30 above, and further in view of Greer et al. (Luminescence. 2002 Jan-Feb; 17(1): 43-74, Review) and Lowe et al. (US Patent 6,132,983).

Szittner et al., Mao et al., Nawotka et al. and Zolotukhin et al. teach LuxA (luciferase) gene from Xenorhabdus, codon-optimized or codon usage of LuxA gene for maximum expression in mammalian cells as discussed above. Szittner et al., Mao et al., Nawotka et al. and Zolotukhin et al. do not teach the use of codon-optimized LuxA gene for making a kit for analyzing gene expression or using IRES promoter for expression.

Greer et al. teach LuxA gene from Photorhabdus luminescens and marine Vibrio harveyi bacteria, as well as eukaryotic luciferase luc and ruc genes from firefly species (Photinus) and the sea pansy (Renilla reniformis), respectively, that emit light in the presence of oxygen and a substrate (luciferin), cloning in a vector having selectable marker, expression using different promoter and enhancer including IRES in cell cultures, individual cells, whole organisms, and transgenic organisms. Greer et al. also teach humanized luciferase i.e. codon-optimized for expressing human cells. Greer et al. do not teach a kit for testing gene expression in cultured cells.

Lowe et al. disclose a luciferase gene encoding protein from Photinus species, cloning in expression vectors having restriction site, promoter and enhancer, host cells. Lowe further disclose a test kit and reagents for carrying out luminescence assay by using luciferase protein to determine the gene expression.

It would have been obvious to one of ordinary skill in the art at the time of the invention was made to combine the teachings of Szittner et al., Nawotka et al., Greer et al and Lowe et al.

to develop a test kit for carrying out luminescence assay to determine the gene expression as disclosed by Lowe et al. by using LuxA gene of Szittner et al. by optimizing the codon usage as taught by Nawotka et al. by using vectors having restriction sites, promoter/enhancer including IRES as well as selectable marker to determine a gene expression in a sample.

One of ordinary skill in the art would have been motivated to develop a testing kit using codon-optimized LuxA gene for determining gene expression in mammalian cell sample to be used in medical research and diagnostics applications.

One of ordinary skill in the art would have a reasonable expectation of success because making a testing kit by using codon-optimized luciferase to determine the gene expression is customary and widely used in the art.

Conclusion

Status of the claims:

Claims 1-4,7-14, 27,28 and 30-33 are pending.

Claims 1-4,7-14, 27,28 and 30-33 are rejected.

No claim is in condition for allowance.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Iqbal Chowdhury whose telephone number is 571-272-8137. The examiner can normally be reached on 9:00-5:00 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ponnathapu Achutamurthy can be reached on 703-272-0928. The fax phone number for the organization where this application or proceeding is assigned is 703-872-9306.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR

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